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The Effect of Antimicrobial Herbal Remedies on Consumer Probiotic Lactobacilli

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The Effect of Antimicrobial Herbal Remedies on Consumer Probiotic *Lactobacilli*

Niana Gunter

Honors Thesis

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Abstract

The purpose of this study was to analyze the effects of common herbal teas on probiotic growth, specifically *Lactobacillus rhamnosus* GG. The degree of inhibition that *Lactobacilli* exhibited upon exposure to herbal tea extracts was examined first via a disk diffusion assay and later through a pour plate well diffusion technique. The extracts examined in this study were from peppermint tea, green tea, chamomile tea, and lavender tea, all of which have research supporting their role in gut and immune function. Measurement data suggested inhibition in some of the experimental groups and the penicillin control. However, through ANOVA and Tukey HSD analysis, no statistically significant findings were revealed. There is evidence to suggest that although there is some degree of inhibition involved between the herbal remedies studied and probiotic bacteria, specifically *Lactobacillus rhamnosus* GG, the inhibition shown does not support the hypothesis that *Lactobacillus* growth is hindered by herbal remedy use. Future researchers can expand this knowledge further by testing other commonly used plant-based remedies.

Literary Review

Purpose

People around the world have increasingly sought over-the-counter solutions and herbal remedies over other prescribed medicinal remedies to maintain general health and treat or prevent minor health issues (Pezzino et al. 2024). Teas, oils, and plant-based herbal medicines are used to treat stomach aches, headaches, insomnia, nausea, and even mild anxiety (Ekor 2014). Herbal remedies have grown in popularity because many medicinal plants contain bioactive compounds with antimicrobial, antifungal, antiviral, and anti-inflammatory properties that may help reduce pain, inflammation, and infection (Mitropoulou et al. 2023) without the side effects or risks of synthetic drugs like antibiotics (Ekor 2014). These properties, although usually very beneficial, do pose a question regarding what other effects antimicrobial compounds could have inside the body. Herbal remedies are not the only over-the-counter or store-bought natural solutions that people use to help regulate their microflora. Probiotic products with live beneficial bacteria have also gained wide acceptance in maintaining gut health and overall well-being in the modern age (World Health Organization 2006). With these two natural solutions being so common, one may wonder how consumer probiotics and herbal teas interact.

This study explores the growth of one of the most common probiotic bacteria, *Lactobacillus rhamnosus* GG (Sánchez et al. 2019), in the presence of herbal remedies with known antimicrobial properties. This project addresses whether herbal teas that are normally considered healthy, such as peppermint, chamomile, lavender, and green tea,

actually impede or inhibit probiotic growth (Swamy et al. 2016). This study also raises the question of what this means in the context of manufacturers who advertise the positive health effects of their products.

Probiotics and Human Health

Probiotics come in many forms, be it through supplemental vitamins or probiotic-rich foods such as yogurt. The Food and Agriculture Organization (FAO) defines a probiotic as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (World Health Organization 2006). These live microorganisms are commonly either *Lactobacilli* or *Bifidobacteria* (Sánchez et al. 2019). Both can be found naturally in fermented foods or can be bought in a supplemental, vitamin form. Foods that contain *Lactobacilli* or *Bifidobacteria* are considered probiotic-rich. Ingesting these foods encourages the growth of these “good” bacteria in the body’s microbiomes, particularly in the gut (Sánchez et al. 2019). The microbes we consider ‘probiotic’ are a natural part of the body’s microflora. Growth of these “good” bacteria is encouraged because of their role in securing gut and intestinal health and their ability to outcompete harmful, toxic bacteria in the gut’s microbiome (Sánchez et al. 2019). Probiotics help in maintaining this balance through several mechanisms. They compete for binding sites and nutrients with pathogens (Figure 1); they produce short-chain fatty acids that lower intestinal pH and create a less favorable environment for harmful bacteria; and they interact with immune cells to reduce

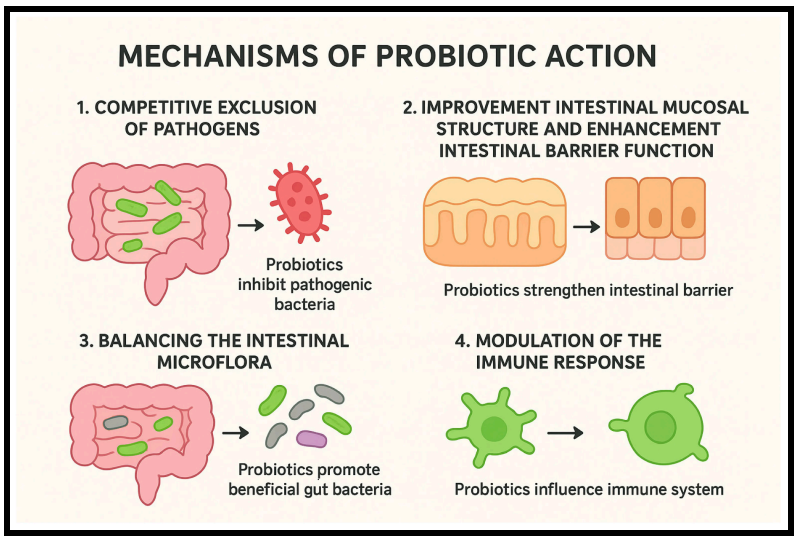


Figure 1. Mechanisms of probiotic action in the human body. Probiotic microorganisms contribute to host health through several mechanisms, including competitive exclusion of pathogenic bacteria (quadrant 1), strengthening of the intestinal mucosal barrier (quadrant 2), balancing of intestinal microbiota (quadrant 3), and modulation of immune system responses (quadrant 4). Through these processes, probiotics help maintain gut microbial balance, enhance intestinal barrier integrity, and support immune function (Idowu et al. 2025).

inflammation (Oliveria 2025). Some strains have even been linked to improving mental health through the "gut-brain axis," the two-way communication pathway between the digestive tract and the central nervous system (Srinivasan 2025) as shown in Figure 2.

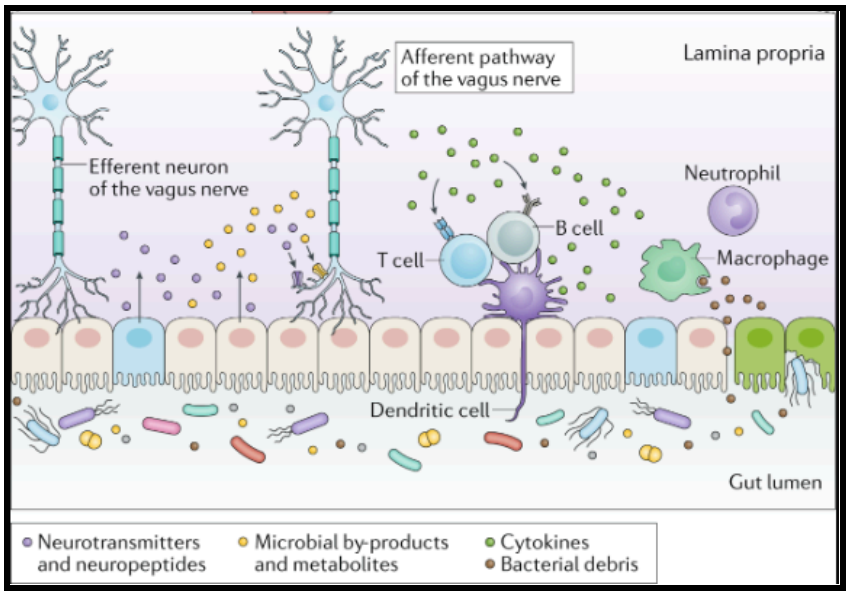


Figure 2. The gut-brain-immune axis and its influence on mental and gastrointestinal health. The gut-brain-immune axis represents the bidirectional communication network between the gastrointestinal tract and the central nervous system. Gut microbiota can influence brain function through the production of neurotransmitters and immune interactions. Within the gut lumen, or cavity, beneficial bacteria and probiotics produce metabolites and signaling molecules that interact with intestinal epithelial cells and immune cells. These interactions stimulate immune responses, including the activation of macrophages, neutrophils, B cells, and T cells, which then release cytokines that can cause inflammation. Neuron signaling is mediated through afferent and efferent pathways of the vagus nerve. Microbials can activate these pathways, transmitting signals from the gut to the brain, while efferent signals from the central nervous system (CNS) can influence the gut's function, producing a call-and-response two-way system. Neurotransmitters and neuropeptides released in this process further contribute to communication between systems. Disruptions to this balance (dysbiosis) can increase inflammatory cytokine production and immune system activation, contributing to systemic inflammation symptoms. This inflammatory state,

along with the associated neural signaling, can influence brain function and has been associated with changes in mood, stress, response, and mental health. Probiotics aid this balance by maintaining gut homeostasis and consequently modifying immune responses, reducing inflammation, and supporting gut-brain communication. In addition, gut dysbiosis and the activation of the adaptive immune system as a result can cause memory B cell activation, resulting in increased allergic reactions (Duke Vertices 2023).

Probiotics function as stabilizers of a microbiome, aiding digestion, immunity, and overall well-being (Hill et al. 2014). You can think of a probiotic as a microbiome manager ingested or–to go along with the analogy–hired to create balance and structure in an unbalanced gut environment. Supplemental probiotic pill capsules contain 1-10 billion colony-forming units (CFUs) of live *Lactobacilli* and *Bifidobacteria* (Oliveria 2025). Although approximately 7 billion CFUs of the bacteria within these capsules are killed within the digestive tract, whether that be in the mouth, throat, stomach, or intestines, enough survive to positively impact ailments such as leaky gut and irritable bowel syndrome (Blaabjerg et al. 2017). Probiotic bacteria have also been found to help regulate the body's microbiomes after antibiotic use, discouraging the growth of harmful bacteria (Britton et al. 2020). Studies have shown that probiotics have also been utilized to strengthen the immune system (Britton et al. 2020), the effects of which can be seen in how the body handles the prevention of allergic diseases and reduces inflammation (Oliveria 2025). By strengthening gut integrity, probiotics can effectively prevent adaptive immune system activation, including cytokine activation and systemic inflammation. Probiotics can also affect the reproductive system, improve skin health, and oral cavity health (Hill et al. 2014). Probiotics support the reproductive

system by maintaining a healthy microbial balance (especially *Lactobacillus),* which helps regulate vaginal pH and prevent pathogenic infections in the genital cavities (Cribby et al. 2008). They can also improve skin health by reducing inflammation as well as conditions such as acne and eczema (Knackstedt et al. 2020). In the oral cavity, probiotics inhibit pathogenic bacteria, reduce plaque formation, and support gum health, which consequently lowers the risk of periodontal disease (Vivekananda et al. 2010).

Beyond their role in maintaining gut microbial balance, probiotics have been studied for their influence on a wide range of physiological and pathological conditions. The gut microbiome interacts closely with the immune system, as well as metabolic processes and neurological pathways. Due to this, disruptions in microbial composition, or dysbiosis, have been linked to gastrointestinal disorders, inflammatory diseases, and mental health conditions. As a result, probiotics are being investigated increasingly for their ability to restore microbial balance and improve overall health for an individual (Figure 3).

One area where probiotics have been widely studied is intestinal barrier dysfunction, often described as increased intestinal permeability or “leaky gut.” The intestinal barrier is formed by a layer of epithelial cells connected by tight junctions, which regulate the movement of substances between cells. Tight junction proteins form a tight seal that allows nutrients to pass into the bloodstream while preventing pathogens and toxins from crossing the intestinal wall (Turner 2009). When these junctions become weakened, bacterial components such as lipopolysaccharides, a component of bacterial cellular walls, can leak into the bloodstream (Figure 3). The

body identifies these components as foreign and can, in turn, trigger the activation of an immune system response, which can lead to inflammation (Bischoff et al. 2014).

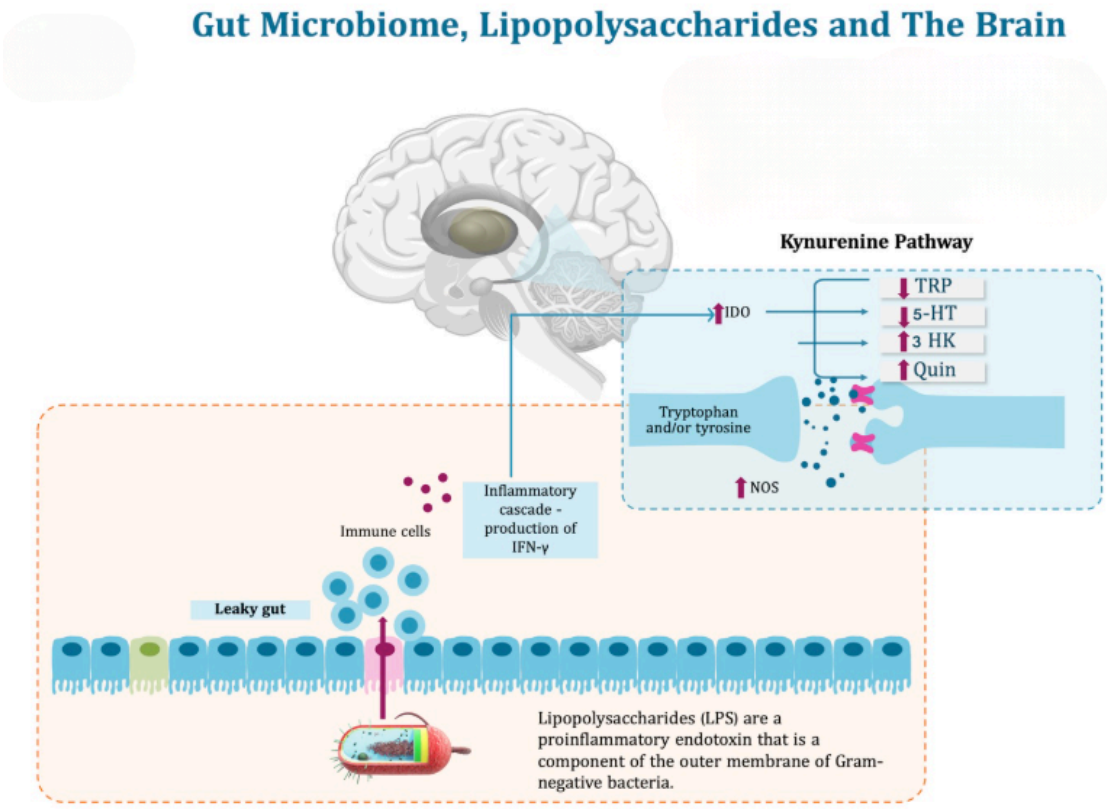


Figure 3. Gut microbiome inflammation and its impact on brain chemistry through the kynurenine pathway. Lipopolysaccharides expelled from bacteria (gram-negative) can cross a weakened or “leaky” intestinal barrier and activate immune cells, triggering an inflammatory response caused by cytokine release (IFN-γ). These inflammatory signals stimulate the enzyme indoleamine 2,3-dioxygenase (IDO), which redirects tryptophan (TRP) metabolism away from production of the “happy” chemical serotonin (5-HT) towards the kynurenine pathway. Serotonin is reduced, and kynurenine metabolites are increased. The rise of nitric acid synthase (NOS) contributes to oxidative stress in the brain. The effects of both contribute to lower mental health outcomes (Turner 2009; Cryan and Dinan 2012; Bischoff et al. 2014; Rege 2019).

The mechanisms by which probiotic organisms have been shown to maintain and restore the intestinal barrier vary. Strains of *Lactobacillus* and *Bifidobacterium* have been shown to stimulate epithelial cells and even increase the expression of tight junction proteins, consequently strengthening the intestinal barrier (Karczewski et al. 2010).

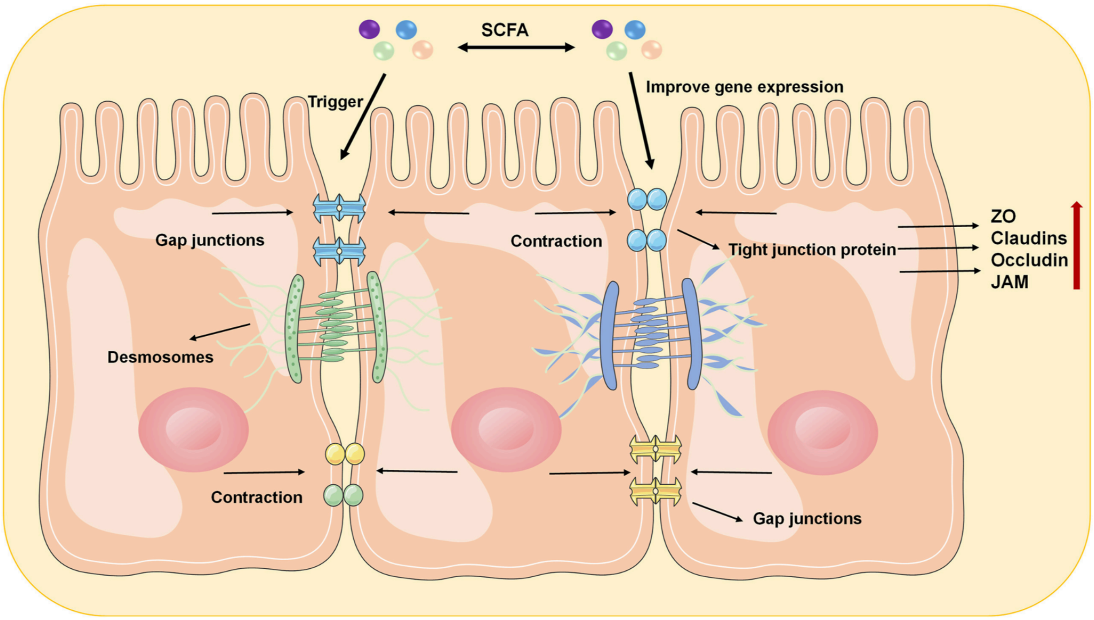


Figure 4. Short-chain fatty acids (SCFAs) enhance intestinal barrier integrity by tightening the tight junctions between them. SCFAs act on intestinal epithelial cells to strengthen barrier function primarily through the reinforcement of tight junctions. SCFA signaling increases the expression of tight junction proteins such as zonula occludens (ZO), claudins, occludin, and junctional adhesion molecules (JAM). This results in tighter sealing between adjacent cells. This tightening prevents the passage of harmful substances and maintains epithelial integrity (Ma et al. 2022).

Additionally, probiotic bacteria can produce metabolites such as short-chain fatty acids, which serve as a source of energy for intestinal epithelial cells (Figure 4). Studies have shown this has also aided in maintaining tight junction stability and reducing inflammatory responses as a result (Ríos-Covián et al. 2016).

Probiotics have also been studied extensively in relation to irritable bowel syndrome (IBS). IBS is a gastrointestinal disorder that is characterized by symptoms such as abdominal pain, bloating, constipation, and diarrhea. The exact cause of IBS is still being studied; many of the patients with IBS that have been studied have shown unstable gut microbiota compared to a patient without IBS (Ford et al. 2014). Clinical studies suggest that certain probiotic strains may reduce IBS symptoms by restoring microbial balance. This consequently decreases intestinal inflammation (Figure 3) and improves overall gut health and nutrient mobility, and cellular organism motility (Didari et al. 2015).

Another growing area of probiotic research involves the gut-brain axis (Figure 2), which refers to the bidirectional communication network between the gastrointestinal tract and the central nervous system. Signals from gut microbes can influence brain function. In particular, this occurs via immune signaling molecules and neural pathways such as the vagus nerve (Cryan and Dinan 2012). Due to these interactions between gut health and neurological pathways, studies have shown gut microbiota to be linked to conditions including anxiety, depression, and stress-related disorders (Simpson et al. 2021).

Certain probiotic strains have been shown to influence the production or regulation of neurotransmitters involved in mood and cognition. For example, some

Lactobacillus and *Bifidobacterium* species can produce or stimulate the production of compounds such as gamma-aminobutyric acid (GABA), serotonin precursors, and dopamine-related metabolites, which play important roles in emotional regulation and stress response (Dinan and Cryan 2017). In clinical studies, probiotic supplementation has been associated with reductions in anxiety symptoms, improvements in mood, and decreased levels of stress-related hormones such as cortisol (Sarkar et al. 2016). For this reason, probiotics that influence neurological pathways are sometimes referred to as “psychobiotics.”

In addition to gastrointestinal and neurological effects, probiotics may also influence immune system regulation. A large proportion of the body’s immune cells are located within the gastrointestinal tract, where they interact continuously with the gut microbiota. Probiotic organisms can stimulate immune responses by promoting the production of anti-inflammatory cytokines and regulating immune cell activity (Hill et al. 2014). Through these mechanisms, probiotics may help reduce chronic inflammation and contribute to immune homeostasis.

Probiotics interact with multiple physiological systems, including digestive, immune, and neurological pathways. As such, their health effects extend far beyond simple digestive support. Understanding the conditions in which probiotics provide benefits is important when evaluating how external factors, including herbal compounds and antimicrobial plant extractants, may influence these essential probiotics’ viability and function.

As research and understanding of probiotics and how they can benefit the body expand, so does their use by consumers, the food industry, and agriculture. The public

is becoming increasingly interested in natural remedies to ailments (Pezzino et al. 2024). As our use of probiotics grows (Oliveria 2025), it is worth investigating the effectiveness of probiotic use when accompanied by other proven herbal methods that often target similar ailments or parts of the body (Oliveria 2025). It is important to explore whether or not probiotics and natural remedies - such as herbal teas or essential oils - work synergistically or antagonistically, as this information is useful to the everyday consumer as well as for companies interested in incorporating probiotics with their products.

Probiotic Organism Selected for Experimentation

Among the various species studied, one of the most researched and commercially applied probiotic strains is *Lactobacillus rhamnosus* GG (LGG). The strain was originally isolated in 1983 from a human intestinal sample and is known for being extremely resistant to the harsh conditions of the stomach (World Health Organization 2006). It owes its success to its ability to adhere to the intestinal mucosa and effectively colonize the gut in competition with other bacteria in the gut microflora (Segers & Lebeer, 2014). Unlike the vast majority of bacteria, which die in the acidic environment of the stomach, LGG is resistant to bile salts and low pH (World Health Organization 2006). LGG is a prime candidate to manage the gut microbiome and restore the dominance of “good” gut bacteria within the body’s microflora.

LGG has been shown to prevent gastrointestinal disorders, like diarrhea, irritable bowel syndrome, and a number of inflammatory gut disorders (Srinivasan 2025). This

probiotic might also enhance immune responses by modifying cytokine production and reinforcing tight junctions between adjacent intestinal cells, helping prevent permeability problems that lead to leaky gut syndrome (Srinivasan 2025). Due to its uses and its lab safety level of BSL-1* LGG is a standard reference organism in probiotic research for studying various interactions between beneficial bacteria and other substances (HiMedia Laboratories 2015).[1]

LGG is grown in de Man, Rogosa, and Sharpe (MRS) medium, which contains carbohydrates, amino acids, and vitamins required for the growth of lactic acid bacteria (HiMedia Laboratories 2015). The optimal temperature for growth of LGG is 37 °C under aerobic conditions, reaching the stationary phase after about 24-48 hours (World Health Organization 2006). This is especially ideal for researchers to study due to the ease of recreating these conditions in the lab.

Antimicrobial Agents Impacting Bacterial Growth and Survival

The way a compound impacts bacterial survival can vary. Some substances can kill bacteria; these are called bactericidal substances. The minimum concentration of a substance that kills bacteria is called the minimum bactericidal concentration (MBC). Other substances can inhibit bacterial growth without eradicating bacterial colonies, a bacteriostatic effect (Pankey and Sabath 2004) and the minimum concentration of a substance that inhibits bacterial growth is called the minimum inhibitory concentration

[1] *Biosafety level (BSL) of a species refers to the safety and clearance levels required to work with the species in laboratory settings. Species with a BSL level of 1 are typically safe for labs with minimal safety procedures. BSL levels of 2 or higher may indicate higher biosafety risks, requiring stricter safety procedures.

(MIC). In other words, the MIC is the lowest concentration of an antimicrobial substance that prevents the bacteria from multiplying. MIC does not show whether or not a substance is necessarily bactericidal; MIC does not require the bacteria to be killed, just inhibited. MIC simply shows that the concentration of a substance is high enough to prevent further bacterial growth. However, upon visual examination, it may seem that the bacterial colony has died. Further testing would reveal if the antimicrobial substance is preventing or inhibiting growth. When working with any antimicrobial substance, it was worthwhile to consider testing to determine the MBC.

Osmoprotectants

Osmoprotectants are small molecules that have the means to help the cell adapt and survive when exposed to osmotic stress (Wood 2011), as shown in Figure 5. Osmotic stress can be high salt environments, drought environments - which go hand in hand - or low salt, high water environments where the osmotic balance across the cell membrane is no longer isotonic (Burg and Ferraris 2008).

There are many different types of compounds that are considered osmoprotectants, such as the amino acids (proline), sugars (trehalose), sugar alcohols (mannitol), and ammonium compounds (glycine betaine) (Burg and Ferraris 2008). These compounds are crucial within a cell to ensure osmotic pressure alone doesn't result in cell death through hypertonic or hypotonic osmosis (Burg and Ferraris 2008).

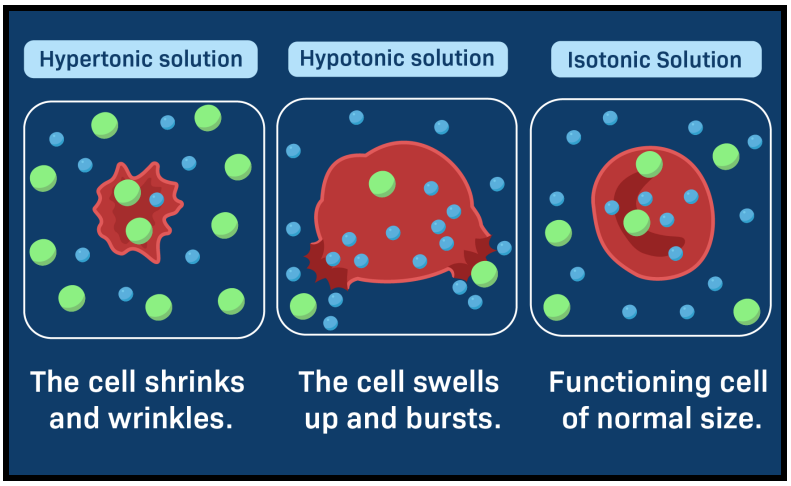


Figure 5. Effects of osmotic conditions on cell structure. Differences in solute concentration inside and outside the cell determine the direction of water movement passively across the cell membrane via osmosis. In a hypertonic solution (far right), water leaves the cell, causing it to shrink. In a hypotonic solution (middle panel), water enters the cell, causing it to swell and burst. In an isotonic solution (far right), water movement is balanced, which allows the cell to maintain its normal size. This is the ideal scenario, as the other solutions lead to cellular death (LabXChange 2021).

Hypertonic osmosis occurs when water rapidly leaves the cell, causing the cell to shrink and ultimately leading to cell death (Wood 2011). This often occurs when the outside environment of the cell has a lower water content and a higher salt concentration (Yancey 2005). Hypotonic osmosis would be when water rapidly enters the cell, often occurring when the salt concentration within the cell is higher than the salt concentration outside of the cell (Burg and Ferraris 2008). Water ions will always flow with their concentration gradient.

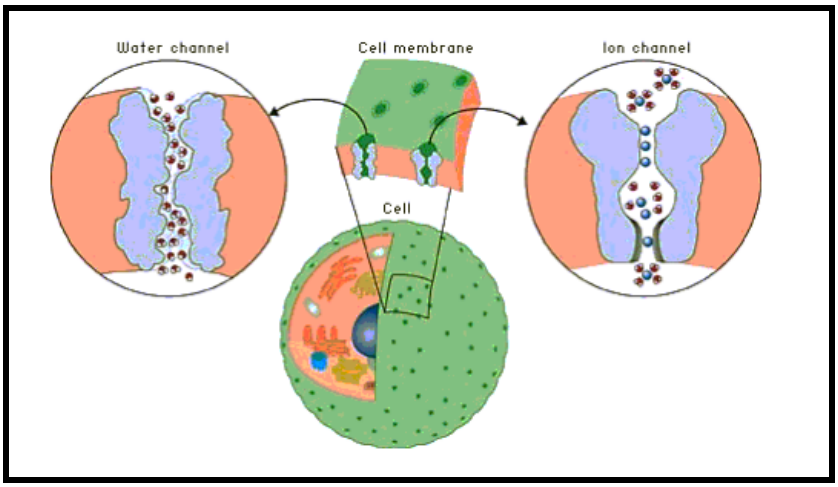


Figure 6. Cellular regulation of osmotic stress through membrane transport of osmoprotectants. Cells maintain osmotic balance themselves through regulating the movement of water as well as solutes across the membrane through membrane proteins such as aquaporins and ion channels. Changes in external solute concentrations can create osmotic stress that leads to water moving in or out of the cell, depending on the hypertonic or hypotonic conditions outside of the cell. To prevent cellular damage due to bursting or excessive shrinking, cells may accumulate compatible solutes known as osmoprotectants, such as proline, trehalose, or glycine betaine. These molecules help stabilize proteins and structures while also maintaining intracellular osmotic balance. They also do not disrupt normal cellular function (Wood 2011; Yancey 2005; Saxena 2009).

A bacterial cell, such as *Lactobacillus*, may not be able to withstand the ion concentration of its environment being significantly higher or lower than the ion concentration inside the cell (Figure 6).

Herbal Remedies in Tea Form

The term “herbal remedy” is a broad term referencing any substance that is used to treat an ailment using plant derivatives (Bent 2008). These plant derivatives have proven effects for either physical medical ailments, such as bee stings or burn wounds (Bent 2008), or even therapeutic/emotional ailments (Sarris et al. 2013). The way these remedies are created and applied can vary widely. Some remedies are ingested after being altered or steeped of its components, such as teas. Others are made into salves. Some are used in powdered form, while others can even involve eating the plant itself. Although the origin of these remedies can be traced back to the traditional medicine practices of a multitude of cultures, including Chinese medicine and traditions (Li and Weng 2017), as well as European herbalism (Petrovska 2012), the use of herbal remedies in modern times has increased exponentially (Ekor 2014). Up to 80% of the world’s population uses herbal remedies in some way or form for therapeutic or medicinal purposes (Ekor 2014).

Peppermint, or *Mentha piperita*, is one of the most commonly used herbal remedies for stomach discomfort, nausea, and general digestive system-related health issues (McKay and Blumberg 2006). Its benefits come mainly from menthol (Table 1), which is the chemical responsible for peppermint's sharp smell and cooling sensation (McKay and Blumberg 2006). Studies have shown that menthol relaxes smooth muscle in the gastrointestinal tract (Grigoleit and Grigoleit 2005). Due to this, it is frequently used in teas to ease cramping or indigestion (McKay and Blumberg 2006). Peppermint also contains natural antimicrobial chemicals that can stop

Table 1. Bioactive compounds present in peppermint, chamomile, lavender, and green tea as well as their associated biological effects. These compounds, found in peppermint, chamomile, lavender, and green tea may contribute to the medicinal properties and potential of herbal teas, but they may also influence microbial growth and probiotic viability (McKay and Blumberg 2006; Srivastava et al. 2010; Cavanagh and Wilkinson 2002; Daglia 2012; Steinmann et al. 2013).

Plant (scientific name)	Major Compounds	Primary Biological Effects	Notes relevant to microbial growth
Peppermint (*Mentha Piperita)*	Menthol, menthone, menthyl acetate	Relaxes gastrointestinal smooth muscle, antimicrobial activity	Menthol can inhibit the growth of bacteria by disrupting cellular membranes
Chamomile (*Matricaria chamomilla)*	Apigenin, bisabolol, chamazulene	Anti-inflammatory, mild sedative effects, digestive relief	Bisabolol and other compounds like it have exhibited antimicrobial effects.
Lavender (*Lavandula angustifolia)*	Linalool, linalyl acetate	Sedative effects, antimicrobial	Compounds can inhibit bacterial growth by disrupting the cellular membrane.
Green tea (*camellia sinensis)*	Epigallocatechin gallate (EGCG), catechins	Antioxidant, anti-inflammatory, digestive support	EGCG disrupts bacterial cell membranes and interferes with the uptake of nutrients into the cell.

the growth of bacteria such as strains of *Staphylococcus* (Soković et al. 2009). These antibacterial effects are partially responsible for peppermint's efficacy in soothing digestive complaints, but also mean that peppermint may interfere with probiotic bacterial substances (Soković et al. 2009).

Similar to peppermint, another commonly used herbal extract is chamomile (*Matricaria chamomilla*), which is best known for its calming effects and is commonly used as a sleep aid or anxiety reducer (McKay and Blumberg 2006; Srivastava et al.

2010). Some compounds in chamomile include apigenin (Table 1), a flavonoid that binds to receptors in the brain associated with relaxation, and bisabolol (Table 1), which has anti-inflammatory and antimicrobial properties (Srivastava et al. 2010). Chamomile tea is also frequently used to help with digestive discomfort in the form of reducing gas and helping with nausea (McKay and Blumberg 2006).

Lavender (*Lavandula angustifolia*) is well known for its use in stress relief, sleep aid, and emotional regulation (Cavanagh and Wilkinson 2002). The components in lavender are linalool and linalyl acetate, both of which contribute to lavender's calming scent and have been shown to have antimicrobial properties (Cavanagh and Wilkinson 2002). Lavender is typically used aromatically or applied topically, but lavender tea is also consumed to reduce tension or soothe digestive issues (Cavanagh and Wilkinson 2002; Koulivand et al. 2013).

Green tea (*Camellia sinensis*) is yet another tea that is often consumed for digestive support, and some people drink it to help reduce bloating, stomach discomfort, or mild nausea (Cabrera et al. 2006). However, the same compounds that make green tea beneficial may also interfere with bacterial growth. Epigallocatechin gallate (EGCG) in particular has been shown to disrupt bacterial cell membranes and interfere with nutrient uptake, which is useful for controlling harmful microbes, but these same effects can be applied to beneficial microorganisms like LGG and *Bifidobacterium* (Steinmann et al. 2013; Daglia 2012).

Research Gaps

There is not much research on the substances or other organisms that inhibit *Lactobacilli*. Most research on *Lactobacilli* is on what *Lactobacilli* themselves inhibit. This makes sense, as the role of a probiotic is usually one of inhibiting potentially harmful bacteria and bacteria that tend to overpopulate and outcompete other beneficial bacteria (Reid 1999). This is very significant because this gap in research could mean we are using *Lactobacilli* inefficiently in our efforts to inhibit other bacteria. The more we know about the effectiveness of *Lactobacilli rhamnosus* GG, both within specific environments and exposed to a variety of different substances and compounds, the more we can learn about the benefits of *Lactobacilli* and other probiotic organisms. This is especially worthwhile for manufacturers who create products using this bacterium. If probiotic-containing products also contain substances that inhibit the *Lactobacilli*, companies may be wasting resources on substances that will ultimately counteract the bacteria and nullify their effects. *Lactobacilli* are especially useful as a probiotic drug within the gut because of their ability to survive, outcompete harmful pathogens, and reproduce successfully within the gut. If the growth of the probiotic bacteria within the gut is being nullified or inhibited, then the consumer would not receive the positive effects of ingesting a product and, therefore, could experience negative impacts such as a weaker intestinal barrier, negative mental health consequences, or harmful bacteria becoming dominant in the gut microbiome.

Introduction

The purpose of this study was to determine the inhibitory effects of favored herbal remedies on a commonly utilized probiotic organism ingested to maintain a healthy gut microbiome. Probiotics and herbal teas are often taken together (Hadjipateras 2024). Some products, such as herbal probiotic teas, even combine probiotic supplements and tea together. This inquiry was selected to test whether natural antimicrobial compounds found in common herbal remedies would disrupt probiotic *Lactobacillus* survival. A benefit of this research is to provide consumers with a better understanding of the biological aspects of ingesting probiotics or probiotic-rich foods alongside standard herbal teas that are antimicrobial in nature. This research is significant because consumers often consider anything deemed “healthy” as something they should ingest without considering how the items they are ingesting may interact with each other. This research may also aid industries that intend to make herbal ‘remedies’ in determining combination approaches that blend those herbal remedies with probiotics. Blended products may be possible that are effective without cancelling effects.

The herbal teas selected were derived from plants with known medicinal properties. The teas chosen for this study were chamomile, lavender, green, and peppermint. Chamomile tea contains compounds that can slow bacterial growth; it is important to test whether these same effects extend to probiotics (Srivastava et al. 2010). Green tea is commonly consumed and is often paired with probiotics for overall gut health (Daglia 2012; Cabrera et al. 2006). Peppermint, chamomile, green tea, and lavender are all common consumer herbal remedies found in tea form with

well-documented medicinal uses (Ekor 2014; McKay and Blumberg 2006; Srivastava et al. 2010). All contain biological compounds with antimicrobial effects, but each herb has a different chemical makeup. Because they are widely used for digestive support, emotional regulation, or relaxation, these teas overlap directly with the reasons many people take probiotics in the first place (Hill et al. 2014). The primary hypothesis was that the *Lactobacilli* would be inhibited by herbal remedies with significantly bactericidal components, such as the EGCG in chamomile or menthol in peppermint, and very little inhibition would be exhibited in the other herbal remedies tested.

Methods

Bacterial Strain, Media, and Growth Conditions

The probiotic bacterium LGG (Bulkprobiotics) was commercially obtained in freeze-dried powder form. To reactivate the bacteria before use, LGG powder (0.1 g) was added to 5 mL of De Man, Rogosa, and Sharpe medium (MRS) broth (Carolina Biological Supply) and incubated at 37 degrees Celsius for 24-48 hours until the vial had visible turbidity and clear aromatic properties that signified *Lactobacilli* growth. A sterile loop was used to streak the revived culture onto Petri dishes (Kindder) containing MRS agar (Weber Scientific) to obtain isolated colonies. After incubation (37 degrees Celsius) for 24-48 hours, a single colony from an agar plate was used to inoculate 5-10 mL of MRS broth to generate an overnight culture for use in the experiment. This step was repeated as needed in order to inoculate enough live bacteria to utilize in the entire

experiment. Sterile water and/or sterile saline (0.85%) were used as a negative control in disk diffusion and well diffusion assays, while penicillin antibiotic disks (Carolina Biological Supply) were used as a positive control in disk diffusion assays only. Sterile blank paper disks were obtained from Carolina Biological Supply.

Preparation of Herbal Teas

Herbal teas were prepared in accordance with typical consumer instructions given by the manufacturer. Tea was measured (2 grams, approximately 1 tea bag) into tea strainers (Jexcull). Peppermint, chamomile, lavender, and green tea (Taylors of Harrogate) were steeped in 250 mL of boiled distilled water for 5 minutes. After cooling to room temperature, each tea for use in disk diffusion assays was prepared at two concentrations: undiluted (1x) and diluted (0.5x), the latter made by mixing equal parts tea and sterile water. Teas steeped for well/pour plate assays were more concentrated (2x). The more concentrated solution was prepared by using half the amount of water to steep the tea.

Disk Diffusion Assay and Interpretation

For the disk diffusion assay, sterile blank paper disks, 6 mm in diameter (Carolina Biological Supply), were dipped halfway into either 1x tea, 0.5x tea, sterile saline (0.85%), or sterile water as to not oversaturate the disk and allow capillary action to

soak the disk entirely. The disks were placed on bacteria-inoculated plates. Each plate contained 3 replicate disks. Penicillin disks (10 U) were used in place of a liquid penicillin source. Bacteria-inoculated plates were incubated and inverted at 37 degrees Celsius for 24-48 hours. After incubation, zones of inhibition were observed and diameters measured in millimeters using a ruler. The average zone size was recorded. Sterile water and/or sterile saline (0.85%) were used as a negative control in disk diffusion and well diffusion assays, while penicillin antibiotic disks (Carolina Biological Supply) were used as a positive control in disk diffusion assays only.

Pour Plate-Agar Well Diffusion Assay, and Interpretation

For the pour plates, tubes of MRS agar media (15 mL) prepared previously were heated to boiling to melt the agar, then cooled until they could be comfortably handled without heat precautions. *Lactobacilli* grown in MRS broth were pipetted (1 mL) into each empty Petri dish. Vials of MRS liquid agar media were poured into the inoculated Petri dishes. Each inoculated Petri dish was then gently swirled to fully incorporate the bacteria into the melted agar media. Once solidified, the wide end of a sterile pipette tip was used to bore wells into the prepared plates. Four wells were drilled per plate. A sterilized inoculation loop was used to remove any excess liquid from the wells. Each treatment (15 µL) was added to the wells via micropipette. The tea treatment groups were tested using 2x concentration. These plates were left at room temperature (~25 degrees Celsius) for 48 hours to incubate. The diameter of each well inhibition zone was measured. A no-zone measurement would be 6 mm in size.

Statistical Analysis

Data was collected by measuring the diameters of each inhibitory zone. The mean was calculated for each specific treatment group. The collected data were analyzed using the ANOVA test of comparative significance with a significance level set as $p < 0.05$ on Graphpad Prism (11.0.0). For further analysis, Graphpad Prism (11.0.0) was used for Tukey post-hoc testing to determine specific significant differences. Data was graphed and visualized on Microsoft Excel and Google Sheets.

Results

Disk diffusion assays were used to measure the inhibitory effects of herbal teas and control treatments on *Lactobacillus rhamnosus* GG. Inhibition zones were observed across both experimental and control groups, with averaged diameters varying depending on treatment (Appendix).

A one-way ANOVA performed with the data from the disk diffusion assays revealed a statistically significant difference between treatment groups ($p=0.0178$). Tukey post-hoc testing revealed that penicillin produced significantly larger inhibition zones than all other treatments ($p<0.001$), while no herbal treatment produced inhibition zones statistically different from water controls (Figure 7). Penicillin, used as a positive control, produced the largest zones of inhibition (Figure 8). The mean inhibition diameter for penicillin was 23.83 mm. Unexpectedly, sterile water negative controls also

produced measurable inhibition zones. Sterile saline (0.85%) negative controls produced a mean diameter of 7.70 mm.

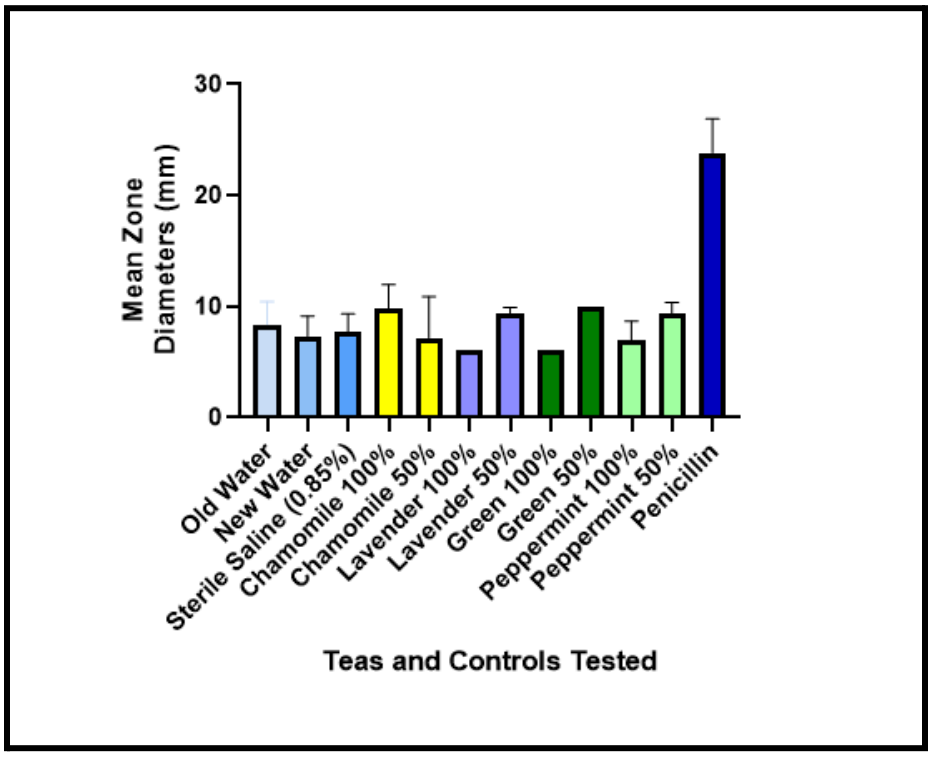


Figure 7. Average inhibition zone diameters for each treatment group were measured using a disk diffusion assay. Herbal tea extracts and control treatments were tested against *Lactobacillus rhamnosus GG* to evaluate antimicrobial effects. Bars represent the mean zone diameter, measured in millimeters, surrounding treatment disks on agar plates. Penicillin served as the positive control and produced the largest inhibition zones, while herbal tea treatments (n=3) and controls (n=9) showed smaller or minimal inhibitory effects. “Old Water” refers to the water used in the initial trial one. “New Water” refers to the water used after the first trial run from a refilled distilled water container.

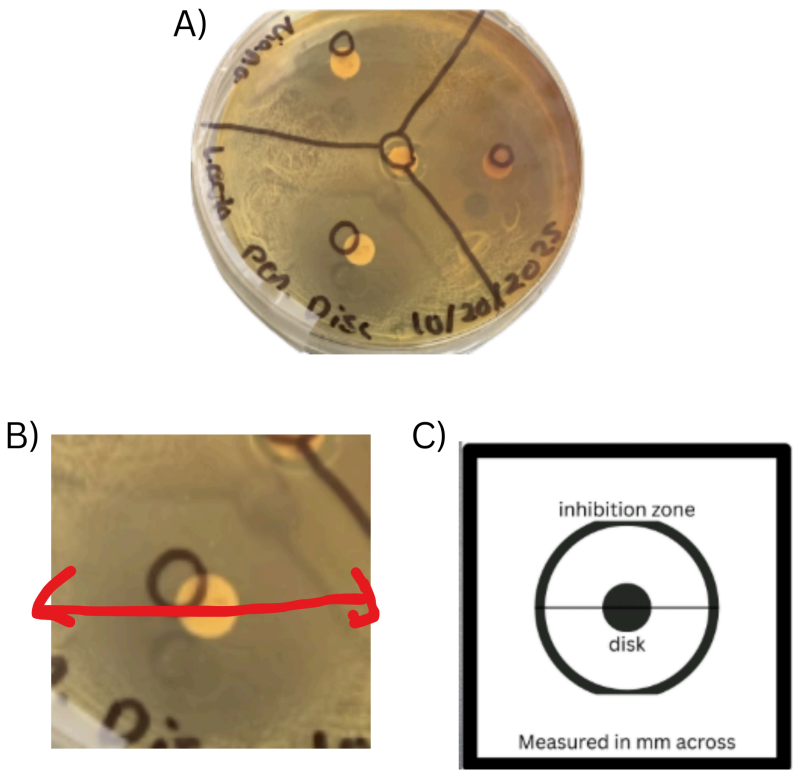


Figure 8. Disk diffusion assay showing inhibition zones produced by the penicillin positive control against *Lactobacillus rhamnosus GG*. A) Clear zones surrounding the antibiotic disks indicate areas where bacterial growth was inhibited. The baseline measurement was 6 mm, as this was the measurement of the disk itself with no measurable zones. B) Penicillin produced the largest zones of inhibition among all treatments, demonstrating strong antimicrobial activity and serving as a positive control for comparison with herbal tea treatments. C) Illustration of zone of inhibition measurements, whereby the diameter of the clear region surrounding the disk, including the disk itself, was measured in millimeters across the center of the zone.

The old water control (sourced from the sterile water jug in trial 1 before being refilled in trial 2) produced a mean inhibition diameter of 8.44 mm. The new water control (refilled sterile water jug) produced a mean diameter of 7.30 mm.

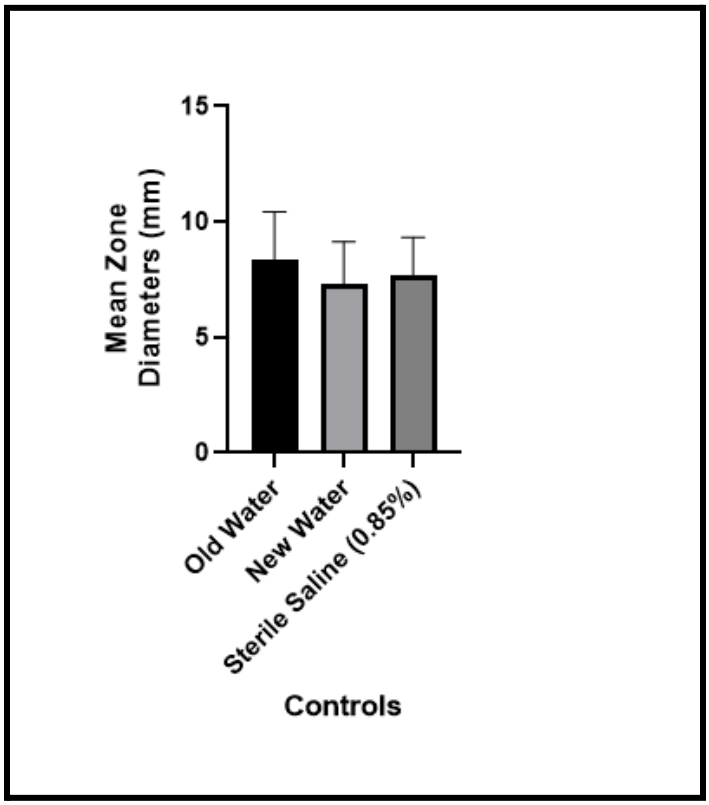


Figure 9. Mean inhibition zone diameters for control treatments measured using a disk diffusion assay. Bars represent the average diameter of inhibition zones (mm) produced by penicillin (n=9) and negative control treatments, including sterile saline (0.85%) (n=9) and two sterile water sources (n=9). The first source of water is indicated as “old water” on Petri dishes, and the second source of water is indicated as “new water” on Petri dishes. Penicillin produced the largest zones of inhibition, while the water and saline controls produced smaller but measurable zones.

Water controls were tested separately against each other for growth and inhibitory comparison due to unexpected results regarding the measured inhibition zones. All treatment groups and controls showed statistically significant p-values against the penicillin control; however, no groups showed statistically significant differences compared to the old water, new water, or sterile saline (0.85%) controls.

Peppermint tea produced measurable inhibition zones at both concentrations tested. Peppermint at 1x concentration produced a mean inhibition diameter of 7 mm, while peppermint at 0.5x concentration produced a larger mean inhibition diameter of 9.33 mm. Chamomile tea produced inhibition zones at both tested concentrations. Chamomile at 1x concentration produced a mean inhibition diameter of 9.83 mm, while chamomile at 0.5x concentration produced a mean inhibition diameter of 7.17 mm. This treatment group was the only group where the 50% concentration was higher than the 100% concentration. However, after Tukey HSD comparison, it was determined that this difference was insignificant with the p-value of 0.5164. Chamomile also showed no other significant p-value when compared to all of the other treatments (with the exception of penicillin). Lavender tea produced different inhibition results depending on concentration. Lavender at 1x concentration produced a mean diameter of 6 mm (the measurement of the disk only), while lavender at 0.5x concentration produced a mean diameter of 9.33 mm. Green tea produced measurable inhibition at 0.5x concentration but not at 1x concentration. Green tea at 1x concentration produced a mean diameter of 6 mm (the disk size only), while green tea at 0.5x concentration produced a mean diameter of 10 mm.

The agar well diffusion test was used to test aerobic and anaerobic growth at greater concentrations (2x) than the disk diffusion test. Data obtained by the pour plate assays were analyzed via ANOVA. The ANOVA determined no significant inhibitory zones, with the average diameters of the treatments measured at 6 mm across which was the diameter of the well.

Discussion

The results of both the disk diffusion assay and the agar well assay suggest that the tested herbal teas do not significantly inhibit *Lactobacillus rhamnosus* GG under the conditions studied, supporting the conclusion that intaking both probiotics and herbal remedies is unlikely to disrupt the gut microbiome. From a consumer standpoint, this indicates that there is minimal risk of gut dysbiosis when probiotics are taken alongside chamomile, lavender, green, or peppermint teas at the concentrations tested. Notably, while it might be expected that higher concentrations would be more inhibitory, the data suggest that more dilute concentrations (0.5x or below) may have produced slightly greater apparent effects, potentially due to osmotic imbalances rather than true antimicrobial activity. The differences, however, were not statistically significant. Chamomile tea was the only treatment group that differed, yet through Tukey HSD statistical testing, the difference in zone measurements was deemed insignificant (0.5164 p-value).

Although there were measurable zones in the disk diffusion assay, these zones were determined to statistically be insignificant via ANOVA and Tukey HSD statistical testing. Chamomile tea in particular showed the greatest zones of inhibition at the 0.5x

concentration during the disk diffusion assay; however, in agar well diffusion, it showed minimal inhibitory effects. The results suggest there is no risk for microbiota dysbiosis by consuming both probiotics and the tested herbal remedies in the concentrations tested. The 2x concentration was utilized as a basis for a “heavy tea drinker.” This approach was used to minimize potential osmotic effects on bacterial cells, as tea contains bioactive compounds such as amino acids and sugars that fall within known osmoprotectant classes and may influence osmotic balance (Luo et al. 2023). The results showed that even in concentrated quantities, the inhibitory effects are different than the diluted treatment. Consumers can intake the herbal remedies tested as well as probiotics without worrying that their gut microbiome may be negatively affected. In addition, herbal remedy production companies can continue supporting the use of their products in conjunction with probiotics.

To eliminate isotonic solution uncertainty that existed with the waters and teas, research could be initiated to find an ideal isotonic solution for the extracting substance so that any growth inhibition on the organism is not a product of overwhelming osmotic pressures (Burg and Ferraris 2008). In these experiments, it can be inferred that the resulting zones of inhibition around the water controls were likely the result of osmotic stress; this would also explain the smaller zones of inhibition around the 0.5x treatment groups.

To determine the nature of inhibition the herbal remedies pose, further testing to determine the effective MIC and MBC should be evaluated. By swabbing and plating from the zones of inhibition on each treatment plate used in this experiment, the growth

or lack thereof can be observed. If the newly inoculated plates show zero bacterial growth, it can be inferred that the effect was bactericidal rather than simply inhibitory.

Further research could also examine whether or not these interactions continue in the presence of digestive proteins. A more thorough examination of the various enzymes found within the human body and how they interact with *Lactobacilli* would lead to a better understanding of how probiotic organisms react to their environment as a whole, giving more insightful results. Beyond enzymes, simulating chewing as the first "barrier" of the digestive tract or modifying pH to simulate the gastric processes could be interesting follow-up experiments.

Other researchers have used ethanol-based solutions to extract nonpolar elements and analyze their effects on the bacteria. As described previously herein, each of the teas investigated have an individual composition involving chemicals reported to be antimicrobial. Investigating each individual chemical and testing them in isolated form against *Lactobacilli* would provide a complete understanding of the individual components in the teas and how they impact the probiotic organism.

The documented scientific research about *Lactobacilli* inhibitory agents is lacking in comparison to the amount of research on gut-host communities and the role of *Lactobacilli* in preventing dysbiosis. More research on *Lactobacilli,* the compounds that inhibit or enhance their survivability*,* and the osmotic conditions suited for survival are all noble goals for future investigation. This recommended future research on the bacteria, their effects on the human body, as well as the best conditions for enhancing growth and survivability of *Lactobacilli,* will allow for better probiotic product development to best benefit human health.

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Appendix

Table 2. Mean Disk Diffusion Inhibition Zone Diameters (mm). Herbal tea extracts and control treatments were tested against *Lactobacillus rhamnosus GG* to evaluate antimicrobial effects. The mean inhibitory zone diameter, measured in millimeters, surrounding treatment disks on agar plates, was recorded. Penicillin served as the positive control and produced the largest inhibition zones, while herbal tea treatments (n=3) and controls (n=9) showed smaller or minimal inhibitory effects. “Old Water” refers to the water used in the initial trial one. “New Water” refers to the water used after the first trial run from a refilled distilled water container. Sterile Saline (0.85%) was also used as a control (n=9) to gain similar control results with additional osmotic protectants. The 100% treatment groups refer to the 1x concentration treatments. The 50% treatment groups refer to the 0.5x concentration treatments.

Treatment	Average of Zone (mm)
Chamomile 100%	9.833333333
Chamomile 50%	7.166666667
Green 100%	6
Green 50%	10
Lavender 100%	6
Lavender 50%	9.333333333
New Water	7.3
Old Water	8.375
Penicillin	23.83333333
Peppermint 100%	7
Peppermint 50%	9.333333333
Saline	7.7
Grand Total	**9.243589744**